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Study of Photoablation of Rabbit Corneas by Er:YAG Laser

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Background and Objective: **This work studied the ablation mechanisms of rabbit corneas by the Erbium:YAG laser. The occurrence of thermal and mechanical damages in the tissue as a function of the laser fluence was also investigated.**
Study Design/Materials and Methods: **The experiments were performed both on enucleated eyes and in vivo. An ultrafast imaging technique was used to investigate the dynamic evolution of the ablation. The treated samples underwent histological and ultrastructural study.**
Results: **A single high fluence laser shot led to the complete removal of the epithelium by a photomechanical effect. In eyes whose epithelium was manually removed, high fluence pulses resulted in evident tears in the stroma, whereas low fluence pulses led to few microns deep incisions, characterized by limited mechanical and thermal damages.**
Conclusion: **The photomechanical action plays a significant role in the ablation of the cornea by Erbium laser. Precise control of the fluence is required to avoid cracking phenomena in the stroma.**

Key words: erbium laser, photoablation, refractive surgery

INTRODUCTION

The ablation of the cornea by the ArF Excimer laser is currently used in reducing myopia and in removing superficial corneal opacities [1–3]. The nonthermal ablation mechanism of the far UV radiation causes minimal damage to the adjacent tissue and removes material with submicron precision [4–6]. However, the clinical usage of Excimer lasers presents several drawbacks. Among them are the difficulty to achieve and preserve a homogeneous beam profile, the high operative cost, and the hazardous handling of toxic gases. A further concern for the employment of the ArF laser in medicine is represented by its potential mutagenic effect, even though there is presently no clinical evidence of any stromal cancer induced in the cornea by the Excimer laser, and the mutagenic risk of the UV refractive surgery is estimated to be very low [7]. These drawbacks argue for research into an alternative laser source for keratectomy.

A wavelength region that has promise for minimizing thermal damage is located ~3 μm where the water presents the highest absorption peak [8]. The first experiments in the MID infrared were made using the HF laser (3 μm) [9,10] and the Raman shifted Nd:YAG laser at 2.80 and 2.92 μm [11]. They gave encouraging results, even though the superior performance of the ArF in terms of precision and limitation of thermal damage was never reached. Moreover, from a technological point of view, none of these systems

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represented an advancement with respect to the Excimer laser.

A significant breakthrough would be achieved if a solid-state laser for keratectomy could be set up. Three main directions are presently being followed in this research field.

A straightforward method concerns the mere substitution of the Excimer laser with a solid-state system that lases at a similar wavelength. Recent progress in the technology of harmonic generation allows a 5th harmonic Nd:YAG laser (213 nm) to be set up. It can be operated with good efficiency and reliability [12–14].

A second direction involves the use of a picosecond (or femtosecond) solid-state laser to generate plasma inside the stroma [15–16]. The plasma results in high absorption of the incoming laser radiation, no matter what wavelength, leading to an effective photoablation. Besides the clinical concerns of the plasma-mediated laser ablation, it is presently unlikely that an ultrashort pulse laser system could be routinely operated in a clinical environment.

The last alternative is represented by the lasers based on the Erbium active ion that emit at ~3 μm (the exact wavelength depends on the host crystal). These systems are attractive for their low cost, simplicity, and reliability. They have demonstrated good efficacy in producing corneal incisions [17–19], although it has been clear since the first experiments that the quality of their incisions in the cornea suffers from the same limitation (mainly thermal damage) already evidenced with the HF laser. Nevertheless, it is the authors' opinion that the ablation mechanism of the cornea by the Erbium laser is not thoroughly understood. Further investigations of the interaction of the laser pulses could lead to a reduction in the tissue damage by a suitable selection of the laser operating conditions.

In this work we have investigated the phenomenological aspects of the cornea ablation by Erbium laser using a time-resolved imaging technique. We have correlated the observations made during the process to the analysis of the histological sections of the treated samples.

MATERIALS AND METHODS

The experiments were performed using a 1-2-3 SEO Er:YAG laser (Schwartz Electro-Optics, Orlando, FL), operated at 1 Hz to avoid thermal lensing inside the laser rod. This system employs an electro-optic $LiNbO_3$ crystal to generate Q-switched pulses 150 ns long (full width half maximum-FWHM). The maximum energy is limited to 25 mJ so as not to overcome the damage threshold of the Q-switch device. The laser beam is nearly diffraction limited with a gaussian profile.

For the experiments, microscope slides were used to attenuate the laser without altering the beam profile. A cylindrical lens of 100 mm-focal length was used to focus the beam on the corneas along a 85-μm-wide (FWHM) stripe. A 3 mm diaphragm was used to crop the gaussian tails of the beam along the unfocused direction. The resulting slit-pattern leads to a monodimensional interaction that simplifies the analysis of the plume by means of the imaging technique.

The time-resolved imaging system used in this study [20] is an evolution of the system used by Puliafito et al. [21] to photograph the cornea ablation by Excimer laser. It relies on an intensified video camera (ICCD-05-SIM Security & Electronic System GmbH, Römerberg, Germany) and on a Nitrogen-pumped dye laser made in our laboratory. The image intensifier gives high sensitivity to the video camera and provides an ultrafast electronic shutter (few nanoseconds). The dye laser emits 1 ns visible pulses that illuminate the sample through an optical fiber. When the Erbium laser fires, the Nitrogen laser is triggered after a variable delay, and the video camera shutter is opened at the same time. The actual exposure time is set by the dye laser pulse duration; nevertheless, the electronic shutter allows operation in normal ambient light, since the background illumination is rejected. One image of the plume is taken at each laser shot. Several delays are scanned in subsequent pulses, allowing a record of the dynamic evolution of the ablation by means of a "sampling" technique. The images are acquired using a stereomicroscope and are digitized and recorded by a frame grabber housed in a personal computer. The speed and the size of the ejected particles were evaluated by means of the time resolved images. The positions of the expanding front of the debris were determined in two images taken within the first 3 μs after the laser pulse, and the particle speed was calculated dividing the mean path by the relative delay of the images. The size of the particles was estimated by means of an image processing software (Image-Pro-Media Cybernetics, Silver Spring, MD).

For this study, nine rabbits were employed. Ten eyes from five rabbits were enucleated a few

minutes before the treatments and kept in the balanced saline solution (BSS-Salf S.p.A., Bergamo, Italy). Four interaction sites were designated in the quadrants of each cornea. Single or multiple pulses of different fluences from 1–3 J/cm^2 were delivered to these sites. Two main conditions have been tested: intact eyes and eyes without the epithelium.

Care was taken to reduce the time elapsed between the de-epithelialization and the laser treatment because the exposure of the stroma to the air may lower its water content and affect the ablation.

Several images of the ablation dynamics were taken at different delays with respect to the laser pulse.

In a following experiment, four rabbits were treated after systemic sedation by means of an intramuscular injection of ketamine 25 mg/kg (ketalar-Parke Davis, Morris Plains, NJ) and topical anesthesia by Oxibuprocanum chloratum (0.4%). After the laser treatment, the animals were euthanized with an overdose of Thiopental Sodium (Pentothal-Abbott, St Laurent, Canada) and the eyes were enucleated. In this experiment, four eyes were treated by single and multiple pulses of different fluences from 1–3 J/cm^2. The remaining four eyes were treated by scanning five pulses over a region 200 μm wide. The rabbit head was kept in a fixed position, whereas the laser beam, focused by the cylindrical lens, was deflected with a tilting mirror in such a way as to focus consecutive pulses to adjacent positions on the cornea (pitch = 50 μm).

Immediately after the treatment, the corneas were sectioned and immersed for 4 hours in a fixative solution (formaldehyde). The samples were embedded in paraffin after dehydration and the sections were stained with hematoxylin and eosin. On the basis of the light microscopy examination, selected samples, after dehydration, were embedded in epoxy resin for the ultrastructural study.

The ablation depth and the extent of the thermal damage were estimated from the histologies. The ablation rates were calculated dividing the crater depth by the pulse number.

RESULTS

Intact Eyes

Single pulse. The first laser shot interacts with the corneal epithelium. The time-resolved images show a disruptive effect that results in the fragmentation of the epithelium in a large amount of particles (Fig. 1). The particles have a variable size up to 50–100 μm and leave the cornea at supersonic speed (≈700 m/s). The measured size indicates that single cells or even cell aggregates are ejected from the cornea. The histological examination (Fig. 2) reveals that a single pulse at a fluence of 3 J/cm^2 leads to the complete detachment of the epithelial layer in the interaction area. However, when the fluence is lower than 3 J/cm^2, in some cases only the superficial epithelial cells are removed; in other cases the epithelium is thermally injured. The cells appear partially swollen and some vacuoles are evident within the cytoplasm.

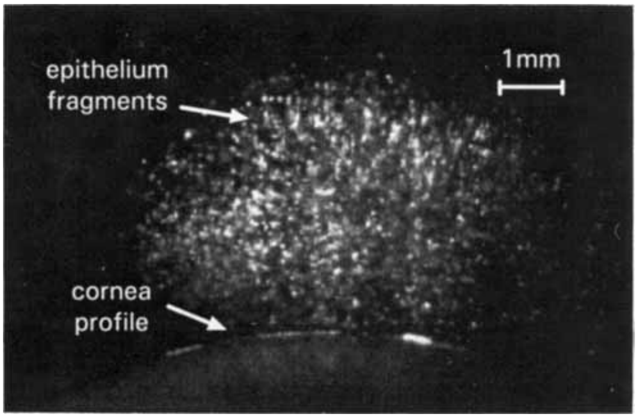
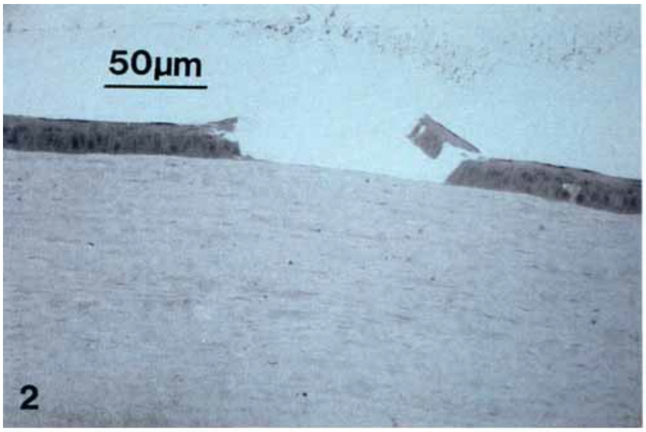
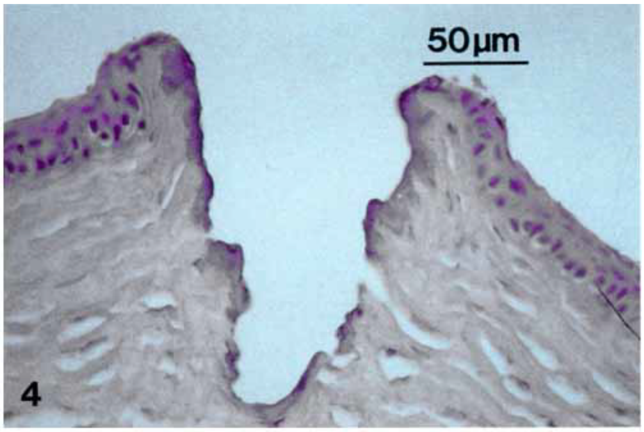


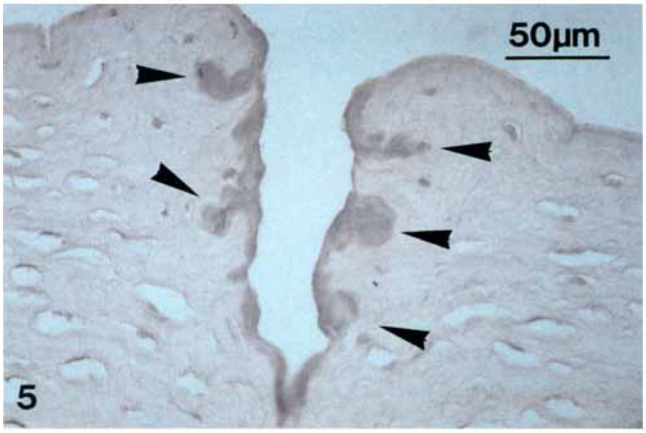
Fig. 1. Fragmentation of the epithelium by a single laser shot. The image was taken 6 μs after the laser pulse. Fluence: 3 J/cm^2.

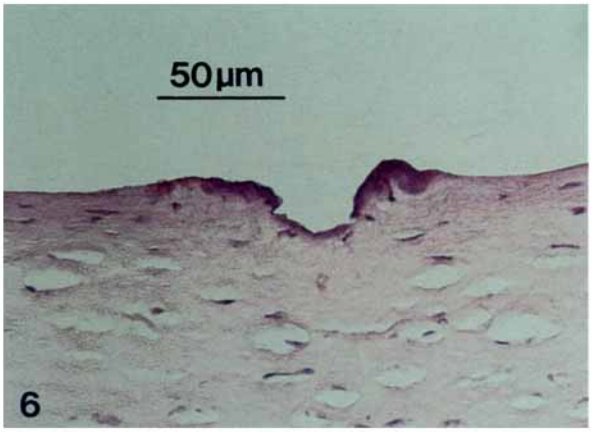
Multiple pulses. Multiple (5–10) pulses remove the epithelium and the anterior stroma as well. The time-resolved images of the plume show that the amount of vapor increases and the content of particles decreases after the first pulse, when the laser interacts with the stroma. In high fluence ablation (3 J/cm^2), some liquid is ejected from the stroma a few microseconds after the laser pulse, as can be seen in Figure 3. The histology of the incisions obtained after 10 pulses (3 J/cm^2) shows a crater 170 μm deep (Fig. 4). The region where the epithelium is removed extends ~30 μm beyond the crater. A 5-μm layer of thermal damage contouring the crater can be observed in Figure 4 as more densely stained tissue.

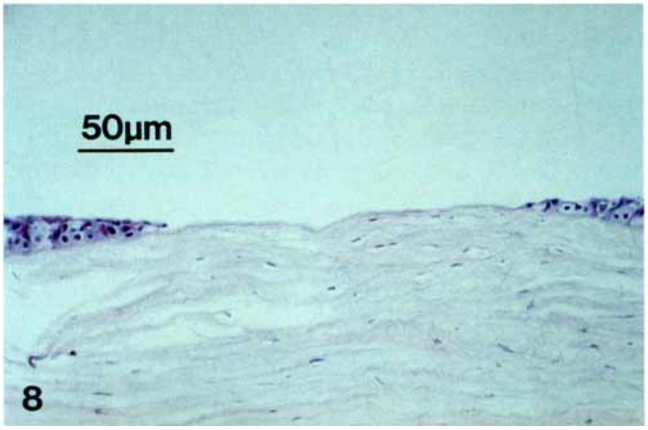
When the fluence is reduced down to 1 J/cm^2, the plume density decreases. As expected, the incisions appear to be smoother than in the case of high fluence, whereas the thermal damage remains comparable.











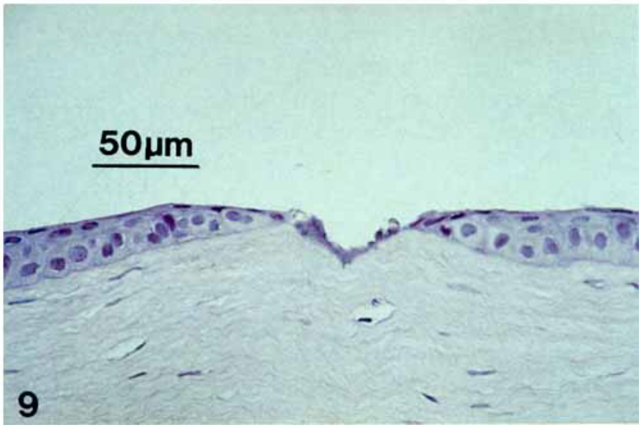


Fig. 2. Histology of rabbit cornea showing the detachment of the epithelium by a single laser pulse having a fluence of 3 J/cm^2.

Fig. 4. Histology of the cornea after 10 laser pulses having a fluence of 3 J/cm^2.

Fig. 5. Incision performed in the stroma with 10 laser pulses having a fluence of 3 J/cm^2. Epithelium was removed by mechanical abrasion. The figure shows some tears contoured by thermal damage (arrows).

Fig. 6. Incision produced in the stroma by five laser pulses with a fluence of 1 J/cm^2. Epithelium was removed by mechanical abrasion.

Fig. 8. Histology showing the region of a rabbit cornea where the epithelium has been removed by 5 laser pulses. The experiment was performed in vivo by scanning the beam over the eye. Fluence: 3 J/cm^2.

Fig. 9. Histology of rabbit cornea after an in vivo laser treatment performed with five pulses having a fluence of 1 J/cm^2.

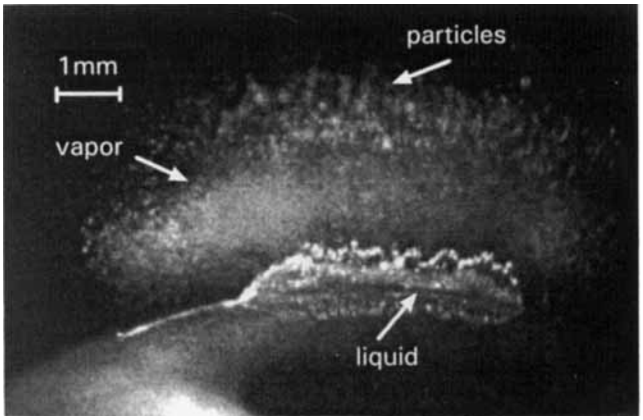


Fig. 3. Expulsion of fragments, vapor and liquid from the stroma 6 μs after the 2nd laser pulse. Fluence: 3 J/cm^2.

Eyes Without Epithelium

The time-resolved images of the plume show fewer particles and more vapor than in the case of the interaction with intact eyes. The ablation rate measured at 1, 2, and 3 J/cm^2 are ~4.8, 7.6, and 17 μm/pulse, respectively, in rather good agreement with the data reported in the literature [17].

The incisions made with the highest fluence (3 J/cm^2) show thermal degeneration (densely stained regions) of the margin of the crater associated with some tears. The tears are sometimes folded, leaving an irregular rim surrounded by a layer of thermal damage (Fig. 5).

The pulses at low fluence (1 J/cm^2) produce smooth and well-shaped incisions without evidence of fractures (Fig. 6). The thickness of the thermal damage in the region surrounding the crater is higher than along the flanks and the bottom of the crater itself.

In Vivo Experiment

The preliminary results achieved in vivo confirm that a single high fluence (3 J/cm^2) pulse removes the epithelium by photomechanical effects, without thermal injury to the stroma. This is confirmed by the ultrastructural examination in which the ablated area is very regular and no homogenization or disorganization of collagen fibers is observed within the anterior stroma (Fig. 7a). The epithelial cells at the rim of the ablated region appear to be normal, even though they are partially detached from the stroma (Fig. 7b).

In samples treated by scanning the beam, the epithelium is removed from a region 175 μm wide (Fig. 8); in the outer region, where the fluence is below the threshold for the occurrence of the complete detachment of the epithelium, this is only partially thinned. At lower fluence (1 J/cm^2), multiple pulses on the same site produce incisions in the stroma (Fig. 9) with a quality comparable to, or even better than the quality achieved in enucleated eyes.

DISCUSSION

Interest in the solid-state lasers at 3 μm has been rapidly growing in the last few years. Among them, the Er:YAG laser is specially promising because its wavelength (2.94 μm) corresponds exactly to the strongest absorption peak of water. Hence, the penetration depth in the tissue presents the lowest value; accordingly, the thermal damage is supposed to reach the theoretical minimum [22]. The Er:YAG laser for the photoablation in medicine was first proposed by Esterowitz [23], and it was soon tested for cornea remodeling [17,18]. Other interesting systems based on the Er active ion are the Er:YSGG laser (2.79 μm), and the CTE:YAG laser (Cr, Tm, Er doped YAG, 2.69 μm).

Some authors estimated that the thermal damage around corneal incisions obtained with the above mentioned lasers in Q-switched regime is rather independent of the wavelength and is limited to few microns [19,24,25]. Our experiments with the Er:YAG laser confirm that the thermal damage is typically confined within a ≈5 μm layer beneath the ablated stroma.

The importance of mechanical effects in tissue removal and in tissue damage during Erbium laser photoablation has been pointed out in several works [18,26,27]. In biological materials irradiated with short laser pulses, stress waves are generated by the fast thermal expansion that follows the absorption of the laser energy (thermoelastic effect) and by the recoil forces originating from the mass ejection [28,29]. Nanosecond pulses having high fluence can generate pressure waves that exceed the elastic response of the tissue and give rise to shock waves [30]. The reflection of an acoustic wave from a boundary where the density changes abruptly can produce a tensile stress. In some cases this stress can exceed the tensile strength of the material, leading to the detachment of a tissue layer, without vaporization. Such a mechanism (photospall) has been proposed by Ready [31] and more recently by Dingus et al. [32] for the photoablation by nanosecond pulses.

The present study demonstrates that me-

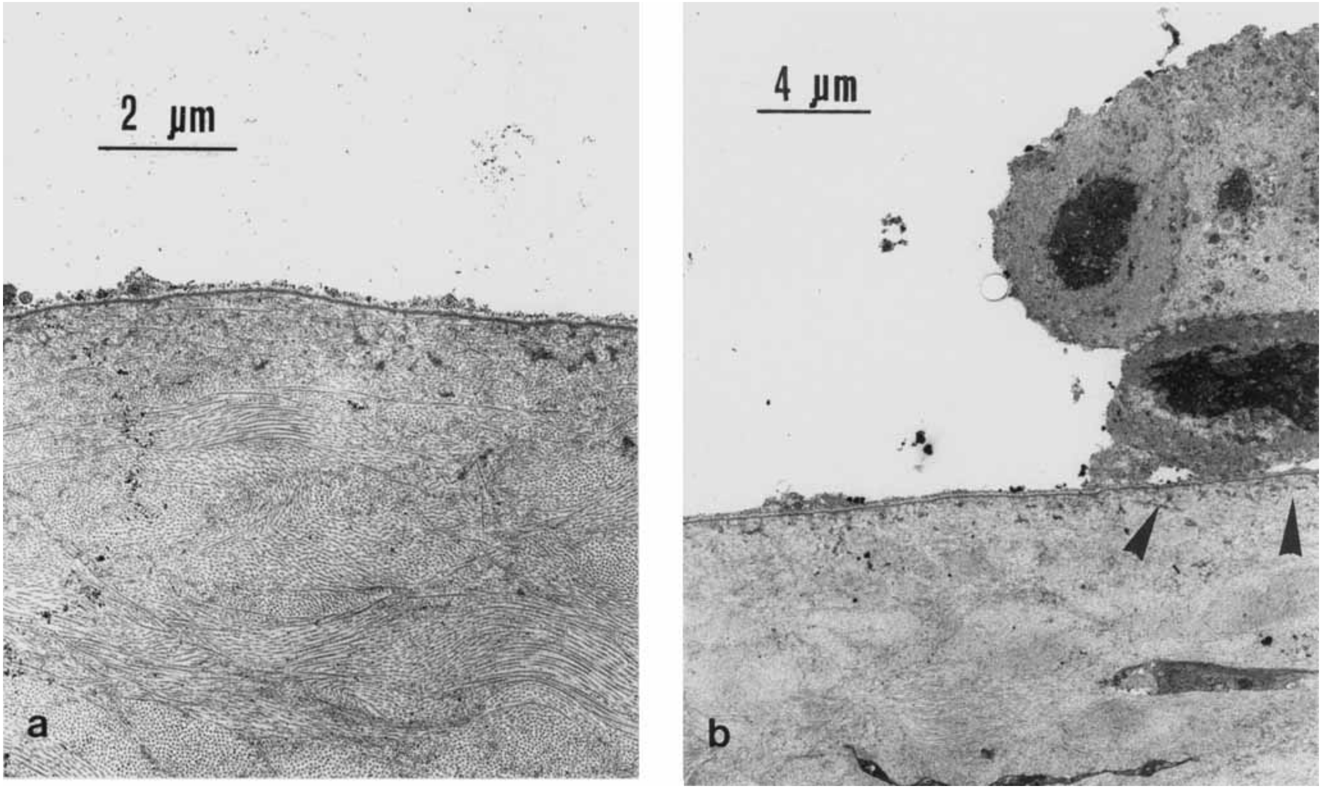


Fig. 7. Ultrastructural study of the anterior stroma after ablation of the ephitelium by a single laser pulse having a fluence of 3 J/cm^2. Central region (**a**) and rim of the lesion (**b**) showing partial detachment of some epithelial cells (arrows).

chanical effects are relevant also in the ablation of the cornea by a Q-switched Erbium laser. The detachment of the epithelium by a single pulse may be ascribed to a sort of photospall mechanism, even though the occurrence of such a phenomenon with the Erbium laser, according to the theory [32], would require pulses shorter than the ones used by the authors. Actually, the layered structure of the cornea very likely leads to an effect similar to the photospall, when the shock wave produced by a Q-switched pulse is reflected at the interface between the epithelial basal membrane and the stroma. Shock waves produced by Q-switched Erbium pulses in water have been previously observed by the authors using the same experimental setup as this study (unpub. obs.).

The ablation by photomechanical effect (photospall) may be regarded as a "cold" mechanism, as it is confirmed by the limited presence of vapor in the time-resolved image shown in Figure 1 and by the complete absence of thermal degeneration of the collagen (Fig. 7a). According to the theory and to our findings, this effect occurs when the fluence is greater than a certain threshold value ($\sim$3 J/cm^2 for the corneal epithelium); otherwise the laser energy leads to a partial vaporization of the epithelium and to thermal damage.

From the above considerations, one might assume that high fluence is always required to minimize the thermal damage. On the contrary, in high energy interactions with the stroma, we found that the forces generated by the fast vapor expansion and by the recoil momentum cause severe fractures in the tissue. When such tears are produced in the stroma, the thermal alteration increases, since some hot vapor penetrates well deep in the tissue bulk along the fractures. The consequences of such a phenomenon appear as a marked dark band contouring the tears in Figure 5. The differences in the structural morphology and in the water content of the epithelium and the stroma may account for the distinct effects observed in these tissues [33]. The larger tensile strength of the stroma and the absence of a boundary layer prevent the spallation from occurring in our experimental conditions. In addition, the high water content favors the tissue vaporiza-

tion, as it appears from the time-resolved image in Figure 3. The presence of some liquefied tissue during the laser interaction with the stroma confirms the photothermal character of the ablation, according to some theoretical models that predict the tissue liquefaction before the vaporization [34,35].

Reducing the laser energy, the ablation rate in the stroma scales more than linearly with the fluence. Low energy interactions (1 J/cm^2) allow a rather precise control of the ablation depth, and preserve the tissue from any mechanical damage. The thermal alterations measured in this condition are comparable to the best results reported in the literature for the Erbium laser. The thickness of the overheated tissue is somewhat increased at the borders of the incisions (Fig. 6), where the ablation does not take place because the fluence of the beam tails is below the threshold. This is an indication for cropping the beam tails along the minor axis of the beam in the focal plane of the cylindrical lens. The greater thermal damage at the boundary of the interaction site also suggest that a further reduction of the fluence could be detrimental. In fact, when the fluence approaches the threshold for ablation, a large fraction of the laser energy is spent to heat up the tissue to the vaporization temperature, whereas only a small portion of the overheated tissue is removed. The excess heat relaxes at the end of the interaction increasing the thermal damage.

CONCLUSIONS

This study demonstrates that for the photoablation by Erbium laser, the fluence and the tissue properties are relevant to discriminate between thermal and mechanical effects.

Tissue ablation by the photospall mechanism was observed for the first time with the Erbium laser. When the conditions for the occurrence of this effect are fulfilled, the laser acts as a cold scalpel leading to a purely mechanical ablation without thermal damage. Presently the photospall could be achieved only in the cornea epithelium by increasing the fluence above a definite threshold, whereas no photospall was observed in the stroma at any fluence. This behavior has been ascribed to the morphological differences of these biological materials and to the relatively long Q-switch pulses used in this study. According to the theory, shorter pulses (hundreds of picoseconds), which could be generated in the mode-locking regime, are required to meet the conditions for the occurrence of the photospall also in the stroma.

The photoablation of the stroma by the relatively long Q-switch Erbium laser pulses takes place mainly by thermal vaporization. In this condition, a relatively low fluence (1 J/cm^2) is required to avoid tissue tears and to minimize side effects.

Even though the Erbium laser cannot presently be proposed for corneal surgery, our results encourage further studies of the interaction mechanisms of ultra short pulses with the stroma.

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